Report for 2004NJ73B: Use of stable isotope ratios of mercury to track and differentiate between sources of mercury pollution

- Conference Proceedings:
  - Kritee K., Bjorn Klaue, Tamar Barkay & Joel Blum, Mercury isotopic fractionation observed during the reduction of Hg(II) to Hg(0) by the bacterial mercuric reductase, presented at The 7th International Conference on the Mercury as a Global pollutant, RMZ Materials and Geoenvironment. Mercury as a global pollutant, Ljubljana, Slovenia (June 2004),Vol. 51, No: 2, 1154-55.
  - Kritee K., Bjorn Klaue, Joel D. Blum and Tamar Barkay, Biological Hg isotope fractionation presented at the 15th International Goldschmidt conference, Idaho. Geochimica et Cosmochimica Acta Vol. 69, Issue 10, Supplement 1, Page A708 (15 May 2005)

Report Follows

Document 2 Project Information: Ongoing project

Problem and Research Objectives:

Mercury (Hg) is a toxic and bioaccumulative trace metal with complex biogeochemistry. Total Hg deposition in the state of New Jersey (NJ) exceeds 600 Kg/yr. But the air emissions of mercury from within NJ do not appear to account for the majority of the deposition in the state. Elemental mercury (Hg^0) has a half-life of about a year, it can travel long distances across the globe before its atmospheric deposition. Therefore, New Jersey Mercury Task Force (NJMTF, 2002) recommended that tools, which can be used to estimate of the relative contribution of in-state sources and out-of-state sources be maintained and enhanced. Once deposited, the speciation (and toxicity) of Hg depends on which transformations of mercury are dominant in that environment. Therefore, the task force also emphasized the need to develop tools to track the fate and transport of Hg in the environment. Stable isotope ratios of Hg can prove to be an important signature buried in the source of Hg and the stable isotope ratios can also be used to study the fate of Hg in a given environment. This project addresses the question: 'Can Hg stable isotope ratios be used to track the fate and transport of Hg at a given site, and distinguish between biological vs. non-biological transformations of Hg?'

Background

Toxicity: From among different species of Hg, methylmercury ($CH_3Hg[I]$ or MeHg) is of the most concern to public health because of its ability to get biomagnified and bioaccumulated (upto 10^7 times) in fishes (Mason *et al.,* 1995; Barkay, 2000). Exposure to MeHg during fetal and neonatal periods effects motor skills such as walking and speech, and may cause mental retardation and so freshwater fish with Hg content of more than 1.5 ppm can should not be eaten (Brigham *et al.,* 2003).

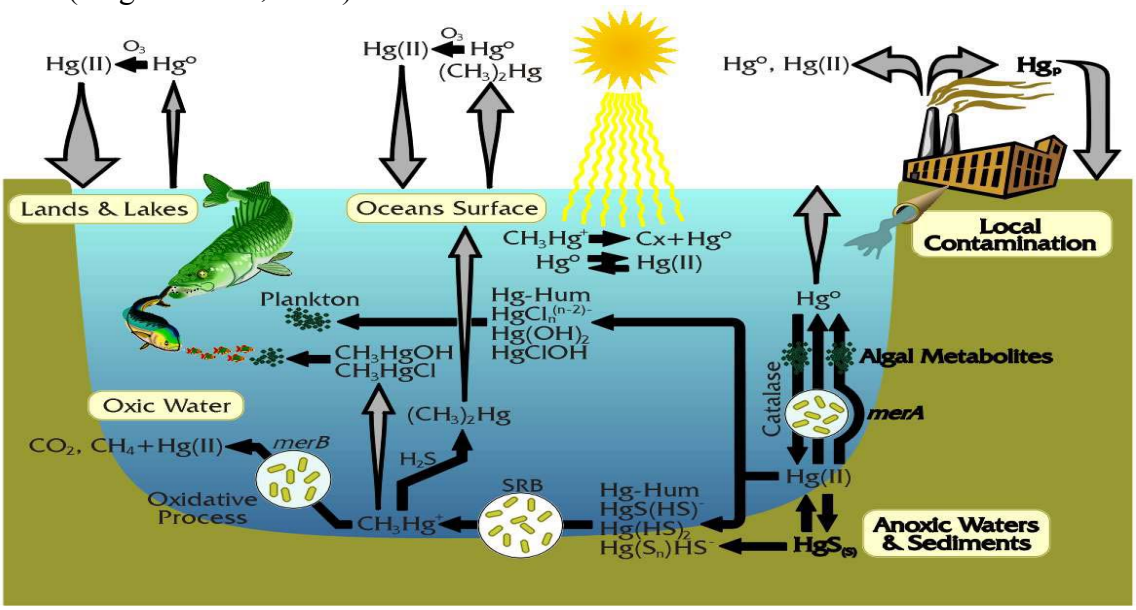


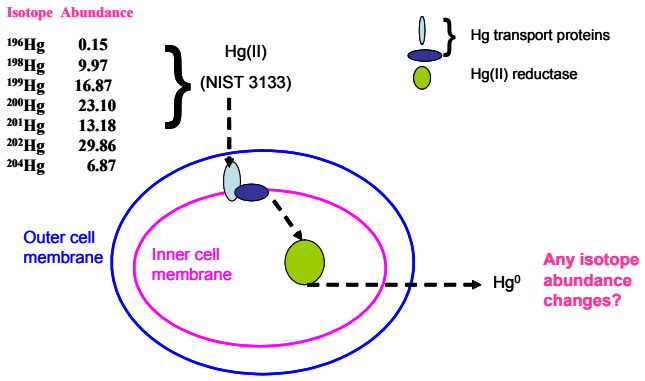
Figure 1. The biogeochemical cycle of Hg. Solid arrows indicate uptake or transformation of Hg and hollow arrows indicate transport pathways. The width of hollow arrows reflects the relative importance of different fluxes. Scheafer *et al.,* 2002.

Need to identify sources of pollution: Since 69-80% of Hg deposition in NJ is contributed by human activities and can be controlled, the NJMTF recommended that NJ adopt strategies to achieve a 65% reduction in air emissions of Hg from within NJ by 2011 (NJMTF, 2002). But even if local input of Hg to our state's atmosphere is decreased, transport of Hg^0 from global sources can continue to pose threat to NJ's ecosystems. Policy formulation and enforcement requires that we know which sources (geogenic vs. anthropogenic; biological vs. non-biological, in-state vs. out-of-state) and which processes (new deposition vs. mobilization of old Hg) are contributing to Hg pollution in a given environment (Fitzgerald, 1993; Rudd, 1995). Unfortunately, the existing probabilistic models for estimating relative contribution of local vs. global sources of Hg predict a highly variable (30-70%) contribution of local sources in NJ and elsewhere (NJMTF, 2002; Rob Mason, *personal communication*).

Need to study fate and transport of Hg: As depicted in Figure 1, once deposited into aquatic environments, Hg[II] is transformed to different species of Hg, both by biological and non-biological processes (Fitzgerald, 1993; Barkay, 2000). Aerobic microorganisms which have capability to make an enzyme called mercuric reductase, reduce Hg[II] to Hg^0. This process leads to loss of Hg from the immediate vicinity of the microorganism but adds to the global pool of gaseous mercury. Anaerobic sulfate reducing bacteria cause methylation of Hg [II] to MeHg by non-specific mechanisms (Barkay 2003). Reduction and methylation can also occur abiologically (Morel, 1998). Humic substances in the soil/sediment can methylate and reduce Hg[II] in the presence of catalysts like Fe and Mn or sunlight. But the relative importance of these non-biological transformations of Hg is a contested issue (Barkay, 2003). It can be extremely useful to determine which pathway (biological vs. non-biological) whether MeHg in a given environment is being synthesized biologically vs. non-biologically, thus helping to direct remediation efforts in an appropriate direction.

Figure 2a. Bacterial Hg resistance proteins

Do these proteins and enzymes coded preferentially reduce lighter isotopes of Hg(II) to Hg^0 compared to heavier ones?



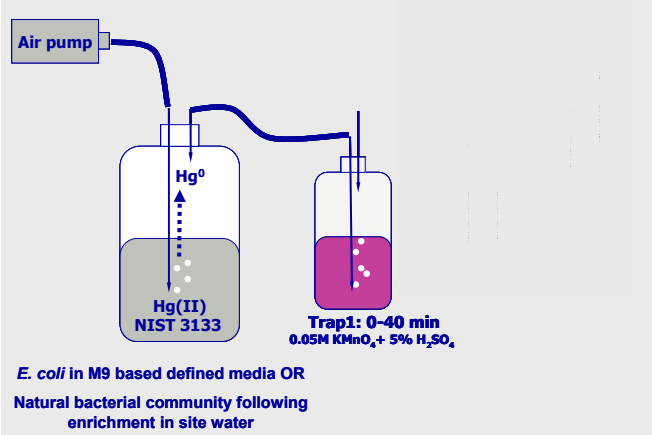
Potential use of Hg stable isotope ratios (HSIR): Stable isotope ratios of sulfur, carbon, nitrogen and lead have been used to recognize the source of pollutant or distinguish between man-made or biological source since the early 1970s. Hg has seven naturally occurring (non-radioactive) stable isotopes and this study intends to explore the possibility of using HSIR to track and differentiate between different sources of Hg. Klaue and Blum (2000) have developed a cold-vapor generation multi collector inductively coupled plasma spectrometry (MC-ICPMS) method, which has allowed them to get consistent high precision Hg isotopic ratio measurements. In order to successfully use HSIR to determine the source of Hg in a given sample, it needs to be determined how can the isotope ratio unique to a source be modified by bacterial processes. Stable isotope ratios can also be used for tracking the fate and transport of Hg in an environment. Determining isotopic fractionation (see below) by pure cultures of Hg transforming microbes will be an important

step in that direction. The knowledge of extent of fractionation by microbial communities can tell us how can the isotope ratios change during Hg's biologically mediated cycling.

Specific objectives: 1) Optimization of experimental setup to determine SIF during reduction of Hg[II] to Hg^0 by a pure culture of bacteria possessing mercuric reductase. 2) Determination of the effect of temperature, concentration of substrate mercurial, the extent of reaction completed and electron donors on SIF during the bacterial reduction 3) Determination of the extent of SIF during reduction of Hg[II] to Hg^0 in a contaminated natural water sample in NJ.

Methods

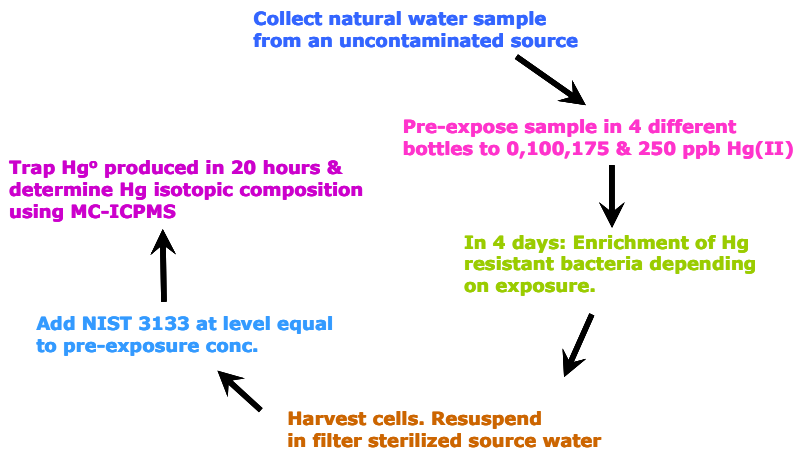
Figure 2b. Simplified schematic of the experimental set up.



Hg(II) reduction by a pure culture NIST 3133 was used as a source of 3 µM (600 ppb) Hg(II). Hg^0 volatilized during the growth of *E.coli*/pPB117 cells at 37^0C (or 22^0C) in M9-based minimal media and was purged into a trapping solution by air stripping (Fig. 2a & 2b). In order to determine the change in isotopic composition as a function of the extent of the reaction, traps were replaced every 30-40 min for a period of 320 min (and every 90 minutes for a period of 900 minutes for the experiment at 22^0C) to collect products corresponding to different stages of the reaction.

Hg reduction by naturally occurring bacteria : NIST 3133 was added to water samples from an uncontaminated source after a 4 day long pre-exposure and Hg^0 produced was purged into a trapping solution (See Fig. 2b & 4). 250 ppb NIST was added to the control given no exposure.

Figure 4. Scheme followed to determine extent of fractionation during Hg(II) reduction by naturally occurring bacteria



MC-ICPMS analysis

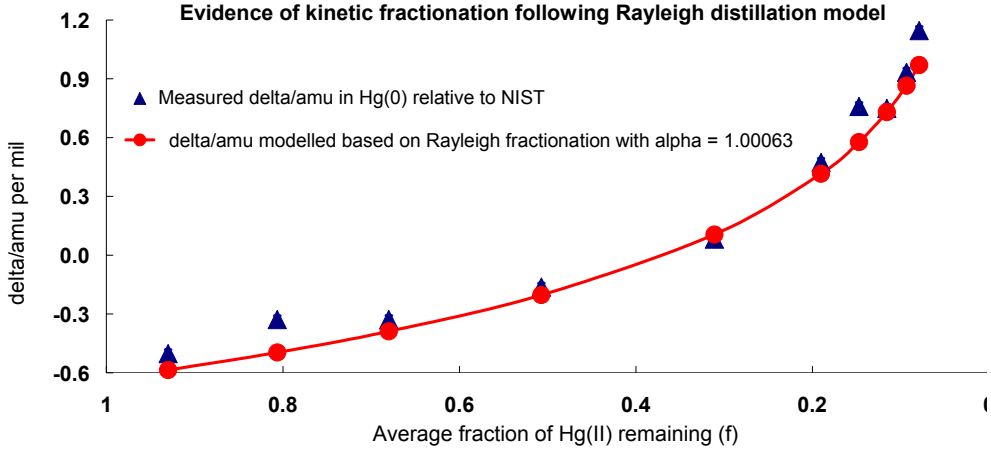
Sample introduction: Cold vapor generation was employed using Sn(II) reduction. The cold vapor sample introduction has a > 99% efficiency and generates a signal of ~600 mV/ppb at a sample consumption rate of 0.75 mL/min. Precision: Fractionation was measured relative to the NIST 3133 Hg standard run before and after

each sample and data are presented as $\delta^{202}Hg/^{198}Hg$ (hereafter $\delta^{202}Hg$). Typical in-run precision of better than ±0.05‰ (2σ) and external reproducibility of δ^{202} between NIST 3133 and a secondary standard was ±0.08‰ (2σ). The kinetic fractionation factor (α) was determined from the results of our experiments using the Rayleigh Distillation Equation: $R_{Vi}/R_{Lo} = (1/\alpha)\, f^{(1/\alpha\, -1)}$

Principle findings and Progress report:

At 37^0C, Hg(II) undergoes mass dependent Rayleigh fractionation (Fig. 3a) with fractionation factor (α) = 1.0006 +/- 0.00005 per amu during its reduction to Hg^0 by *E. coli*.

Figure 3a.
Evidence of kinetic fractionation following Rayleigh distillation model



At 22^0C, preliminary estimation indicates that α ~ 1.0015

For Manipulated naturally occurring bacteria: When Hg^0 was produced after being pre-exposure to Hg(II) conc. of 250 & 175 ppb: 100% of surviving bacterial cells were Hg resistant & α ~ 1.0006 (similar to pure culture) was observed. But at low or no pre-exposure: Much lower % of total cells (10%) were Hg resistant & lower extent of fractionation (Fig. 5a and 5b) was observed.

Conclusions:

- Systematic Hg stable isotope fractionation does happen, both in pure cultures of bacteria and naturally occurring bacterial consortia!

- Hg is the heaviest metal for which biological fractionation has been detected to date. In spite of the reduced % mass spread of its isotopes and increased molecular weight, the extent of fractionation found lies in the same range as for much lighter elements (Table 1).

Table 1. Comparison* of the extent of fractionation observed for Hg with other redox-sensitive elements undergoing fractionation[5,6].

	Avg. Mol. Weight	% mass spread	Maximum Range of δ (‰/amu)	Maximum reported α/amu
Fe	56	7	2**	1.0015**
Se	80	10	3#	1.003#
Mo	96	8	1.7##	1.002##
Hg	**200**	**4**	**2**	**1.0015**

* This is a crude comparison & does not include fractionation due to amplifying processes such as iterative distillation or chromatgraphy.

** Maximum range of isotopic variation (relative to standard) reported for low temperature processes occurring either in nature & under laboratory conditions. Eg. $\delta^{56/54}$Fe in natural samples varies from ~-3 to +1 making the max. range ~2‰/amu[5]. Max. α for $^{56/54}$Fe is 1.003 for non biological redox eqm. of Fe(III) and Fe(II)[5].

Max. ε ~13‰ for ^{80}Se/^{76}Se during various Se transformations (or 3.25‰/amu). ε = 1000*(α -1)(Johnson & Bullen[6]).

$\delta^{97/95}$Mo varies between -0.9 to +2.5 for natural samples (Anbar[6]).

- Use of Hg isotope ratios for identifying sources and sinks, in situ pathways leading to its toxicity, and/or the nature and evolution of redox reactions in both modern and paleo environments is plausible.

- Future work will determine how the change in physico-chemical parameters (T, pH, e-donor etc.) can change the extent of fractionation during Hg(II) reduction and other Hg transformations.